Intestinal microbiome analysis

The 16S Microbiome NGS Assay

A powerful end-to-end solution combining library preparation and bioinformatic analysis

Intestinal microbiome and the 16S rRNA gene

The human microbiome inhabits nearly every surface of our body and consists of a plethora of bacteria, which outnumber the cells in the human body. The composition and diversity of the human gut microbiome have been linked to a wide range of phenotypes in health and disease.

Why testing?

Constituting approximately 1–3% of our body mass, bacteria aid important metabolic pathways. Reduced activity or imbalances in the gut microbiome have been associated with a variety of conditions, including obesity, inflammatory bowel disease (IBD), type II diabetes (T2D), fatty liver disease, and neurological disorders (e.g autism, multiple sklerosis).[1-5]

Moreover, information about bacteria living in the human gut is growing at a tremendous rate.
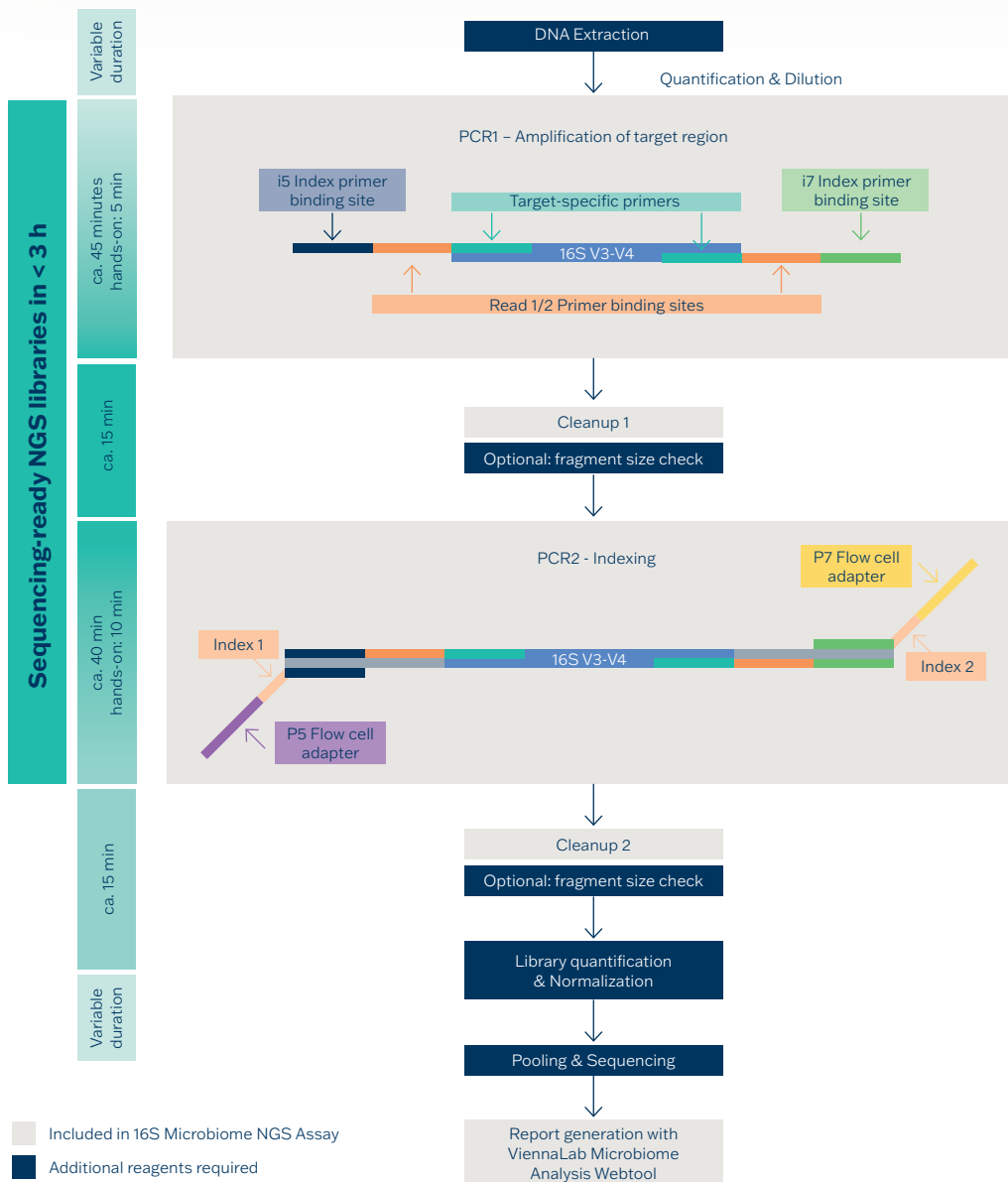
What is being investigated?

Targeted amplification of variable regions V3-V4 of the 16S rRNA gene provides a quick and easy way to assess the microbial composition in the human intestine. The V3 and V4 regions jointly bear the highest variability between different bacterial species rendering these regions well-suited for the in-depth analysis of bacterial richness and constitution. The ViennaLab 16S Microbiome NGS Assay in conjunction with the ViennaLab Microbiome Analysis Webtool enables for species-level classification of bacteria colonizing the human gut.

BioVendor Group **NGS**

Key features

– Targeted library preparation plus bioinformatic analysis
– Species-level classification of bacteria in the human gut
– From extracted bacterial DNA to ready-to-sequence libraries in < 3 hours
– Dual-indexed libraries allowing for large-scale pooling
– Compatibility with Illumina sequencing platforms

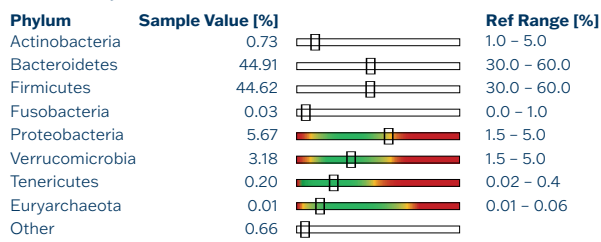## Workflow and timeline of the ViennaLab 16S Microbiome NGS Assay

References:

[1] Turnbaugh PJ et al. A core gut microbiome in obese and lean twins. Nature 2009;457(7228):480-4
[2] de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol 2016;13(1):13-27
[3] Qin J et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012;490(7418):55-60
[4] Abu-Shanab A, Quigley EM. The role of the gut microbiota in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2010;7(12):691-701
[5] Hsiao EY et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013;155(7):1451-63
[6] Ounit R et al. CLARK: fast and accurate classification of metagenomic and genomic sequences using discriminative k-mers. BMC Genomics 2015;16:236
[7] Quast C et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl Acids Res 2013;41(D1):590-6
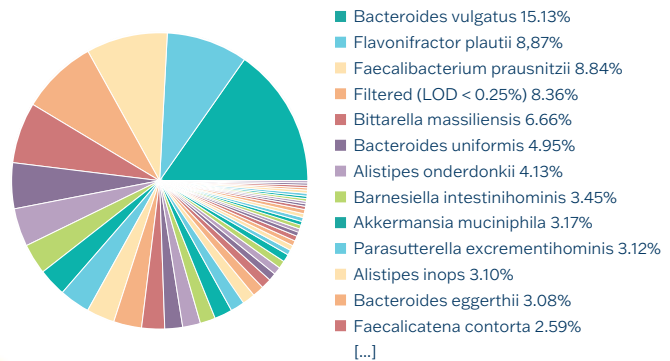
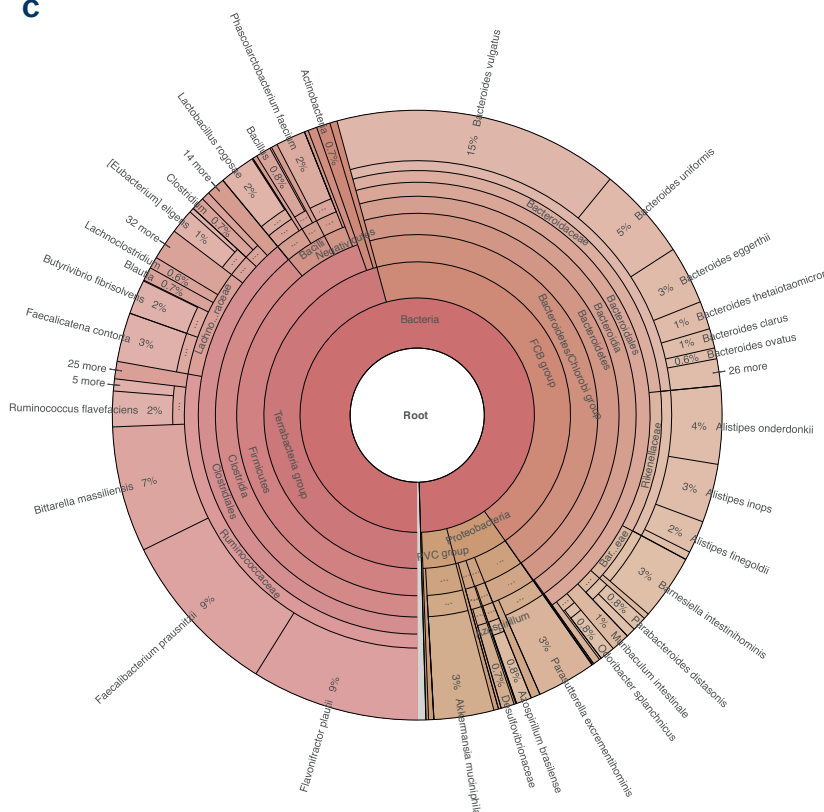# Reporting with the ViennaLab Microbiome Analysis Webtool

A

Bacterial Phyla

Phylum	Sample Value [%]		Ref Range [%]
Actinobacteria	0.73		1.0 – 5.0
Bacteroidetes	44.91		30.0 – 60.0
Firmicutes	44.62		30.0 – 60.0
Fusobacteria	0.03		0.0 – 1.0
Proteobacteria	5.67		1.5 – 5.0
Verrucomicrobia	3.18		1.5 – 5.0
Tenericutes	0.20		0.02 – 0.4
Euryarchaeota	0.01		0.01 – 0.06
Other	0.66		

B

- Bacteroides vulgatus 15.13%
- Flavonifractor plautii 8,87%
- Faecalibacterium prausnitzii 8.84%
- Filtered (LOD < 0.25%) 8.36%
- Bittarella massiliensis 6.66%
- Bacteroides uniformis 4.95%
- Alistipes onderdonkii 4.13%
- Barnesiella intestinihominis 3.45%
- Akkermansia muciniphila 3.17%
- Parasutterella excrementihominis 3.12%
- Alistipes inops 3.10%
- Bacteroides eggerthii 3.08%
- Faecalicatena contorta 2.59%
  [...]

C

ViennaLab 16S Microbiome Analysis Webtool

A. Composition of the patient's gut microbiome at the phylum level as compared to established reference ranges (green area of the color bar).

B. Microbiome composition at different taxonomic levels including phylum/class/order/family/genus and species-level classifications (shown).

C. Sunburst (Krona) diagram for the interactive review of microbial composition across different taxonomic levels. Examples have been taken from the ViennaLab Microbiome Analysis Webtool, which allows for the online review of the results, the generation of an informative report and the download of the entire species-level abundance dataset.

The ViennaLab **Microbiome Analysis Webtool** is an easy-to-use solution providing an intuitive workflow for the bioinformatic analysis of 16S NGS microbiome data and the informative visualization of computed classification results.

Raw sequencing data files can be uploaded directly to the webtool and are automatically analyzed with an optimized pipeline utilizing the CLARK sequence classification system in combination with the SILVA high-quality ribosomal RNA database.[6-7]

For easy cross-referencing, the generated taxonomic output adheres to the NCBI classification nomenclature. Additionally, the pipeline provides diversity statistics based on species-level abundance, informative summary tables and graphical output to facilitate the rapid review and comprehensive evaluation of the microbial composition in the analyzed samples. Investigators are also able to download the results in a raw format and perform subsequent downstream analyses according to their specific needs.

Kit components and order information

16S Microbiome NGS Assay
Library Preparation Kit for 96 libraries

RUO

Kit components [REF 9-131]

Activation code	100 bioinformatic analyses
Magnetic Beads	1x 13ml
Box "16S Master Mix 2x"	⬤ 5x 1ml 16S Master Mix 2x
Box "PCR1 V3-V4 Primer Mix"	🟡 1x 400µl MIB1 - V3V4 Mix
Box "PCR2 Indexing Forward Primers **Set A**"	🔵 8x 30µl MIB2A-F1 – MIB2A-F8 Primer
Box "PCR2 Indexing Reverse Primers Box 1/2 **Set A**"	🟣 8x 30µl MIB2X-R1 – MIB2X-R8 Primer
Box "PCR2 Indexing Reverse Primers Box 2/2 **Set A**"	🟣 4x 30µl MIB2X-R9 – MIB2X-R12 Primer

Kit components [REF 9-132]

Activation code	100 bioinformatic analyses
Magnetic Beads	1x 13ml
Box "16S Master Mix 2x"	⬤ 5x 1ml 16S Master Mix 2x
Box "PCR1 V3-V4 Primer Mix"	🟡 1x 400µl MIB1 - V3V4 Mix
Box "PCR2 Indexing Forward Primers **Set B**"	🔵 8x 30µl MIB2B-F9 – MIB2B-F16 Primer
Box "PCR2 Indexing Reverse Primers Box 1/2 **Set B**"	🟣 8x 30µl MIB2X-R1 – MIB2X-R8 Primer
Box "PCR2 Indexing Reverse Primers Box 2/2 **Set B**"	🟣 4x 30µl MIB2X-R9 – MIB2X-R12 Primer

Kit components [REF 9-133]

Activation code	100 bioinformatic analyses
Magnetic Beads	1x 13ml
Box "16S Master Mix 2x"	⬤ 5x 1ml 16S Master Mix 2x
Box "PCR1 V3-V4 Primer Mix"	🟡 1x 400µl MIB1 - V3V4 Mix
Box "PCR2 Indexing Forward Primers **Set C**"	🔵 8x 30µl MIB2C-F17 – MIB2C-F24 Primer
Box "PCR2 Indexing Reverse Primers Box 1/2 **Set C**"	🟣 8x 30µl MIB2X-R1 – MIB2X-R8 Primer
Box "PCR2 Indexing Reverse Primers Box 2/2 **Set C**"	🟣 4x 30µl MIB2X-R9 – MIB2X-R12 Primer

Note: REF 9-131, 9-132, 9-133 differ only in Indexing Primer Sets. If you plan to sequence more than 96 samples on one flow cell, please order kits with different sets (e.g. Set A / REF 9-131 and Set B / REF 9-132). Make sure that all individual libraries sequenced in the same pool have a unique indexing primer combination.

MANUFACTURER:

DISTRIBUTOR:

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